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LIFE HISTORIES OF LOTIC MAYFLIES (EPHEMEROPTERA) IN AN OZARK STREAM: INSTAR DETERMINATION AND VOLTINISM

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Abstract.—Life history studies were conducted on four commonly occurring species of mayflies from an Ozark stream in Missouri. A multivariate statistical technique, using a suite of ln-transformed body measurements of *Stenonema mediopunctatum* (McDunnough) (Ephemeroptera: Heptageniidae) nymphs, discerned at least nine instars during the sampling period. This technique provided greater resolution in discriminating among instars than using raw data or head capsule widths alone. Further, *S. mediopunctatum* was determined to be univoltine in these streams, as were *Caenis hilaris* (Say) and *C. latipennis* Banks (Ephemeroptera: Caenidae). *Tricorythodes curvatus* Allen (Ephemeroptera: Leptohyphidae) apparently was multivoltine.

Key Words: Ephemeroptera, life history, voltinism, instar

The shift in ecological research from descriptive natural history to more quantitative approaches requires that life history and life cycle information constitute a larger part of studies dealing with community-level processes (Rosenberg 1979); however, life histories of most species of aquatic insects are not known in detail (Richardson and Tarter 1976). This type of information is important for a better knowledge not only of the insects themselves, but of their distribution and ecology. Mayflies are an important component of aquatic ecosystems and the life histories must be determined in order to have a complete understanding of their functions in aquatic ecosystems (Kondratieff and Voshell 1980). Further, knowledge of the number of instars for each mayfly species or population, and how this information correlates with environmental factors, will lead to a better understanding of their biology (Fink 1982, 1984), especially when describing life histories, life cycles, or energy budgets (Benton and Pritchard 1988).

The nymphal lifespan of temperate mayflies varies from 7–13 days (Gray 1981) to approximately 2.5 yr, depending on the species and geographic location of the population (see Brittain 1982, Sweeney et al. 1995). Instar number, which is variable in mayflies (Brittain 1982, Fink 1982, 1984), ranges from 10 to 50, with most species in the range of 15–25 (see Brittain 1982, Butler 1984). Further, the number of nymphal instars is not constant for a particular species, but may vary with sex and environmental conditions, such as temperature and nutrition, during development (Brittain 1990). Although voltinism has been determined for many species, the number of instars has been published for only a few. For example, the number of instars for *Stenonema canadense* Walker is between 40 and

45 (Ide 1935), *Ephemera simulans* Walker approximately 30 (Ide 1935), *Ameletus celer* McDunnough 21 (Benton and Pritchard 1988), and *S. modestum* (Banks) 14–15 in the overwintering brood (Kondratieff and Voshell 1980).

Although mayflies characteristically are heterogeneous in growth, morphological development, and instar number (Fink 1982), studies of growth and development often rely on body measurements. An early method, introduced by Ide (1935), was based on the change in segmentation of the caudal filaments. The number of instars that a fully grown nymph has passed through may be estimated by counting the number of segments of the caudal filaments. However, it is difficult to obtain specimens from the field with intact caudal filaments, particularly in a quantified manner. A commonly used metric for mayflies and many other insect taxa is that of head capsule width (e.g., Kondratieff and Voshell 1980), which after direct measurement, are categorized into size classes. The most commonly used methods for mayfly instar determination are rearing, with direct observation of instars, and the simple frequency method, in which instars are indirectly determined by plotting the number of individuals per size class (Fink 1984). Another popular method is the Janetschek method which requires the calculation of a moving average of each simple frequency size class. However, head capsule width, the simple frequency and Janetschek methods, as well as others including the Cassie method and Dyar's Law, have been suggested as unreliable (Fink 1984). In a study that examined the utility of six mensural characters of *Ephemera danica* Muller (Ephemeridae) for determining growth and voltinism, size classes were established based on head capsule width (Aguayo-Corraliza et al. 1991). However, the data were not used to approximate instar number. In that study, only wingpad length was shown to exhibit allometric growth; whereas, body, head, and leg lengths and widths exhibited isometric growth.

Among mid-temperate mayflies, univoltine life cycles are the most common (Brittain 1990), whereas tropical and subtropical populations can deviate from this pattern with shorter, aseasonal life cycles (e.g., Nolte et al. 1996). The duration of a life cycle depends in part on factors that influence growth and development in all life stages. Also, genetic constraints that limit rates of these processes may exist (Butler 1984). Although thermal regime can have a major influence on voltinism of aquatic insects, life history patterns are the result of a complex bioenergetic interaction between temperature and food abundance and quality (Anderson and Cummins 1979, Sweeney 1984).

The family Heptageniidae is widely distributed throughout the Holarctic region and comprises a diverse component of benthic communities in Oriental (Dudgeon 1996) and Palearctic streams. More specifically, heptageniid nymphs of the genus *Stenonema* frequently are the most abundant benthic insects in streams and rivers of eastern North America (Bednarik and McCafferty 1979). The elucidation of the complete life cycle and life histories of these mayflies and other aquatic insects in the stream community is fundamental for a complete understanding of community dynamics (Richardson and Tarter 1976). This research was conducted to determine if using a suite of nymphal morphological characters with multivariate statistical analyses would provide greater resolution than other commonly used techniques in estimating the number of instars for a given mayfly species. Further, to examine voltinism, head capsule widths were taken and size-frequencies were calculated for four lotic mayfly species.

Materials and Methods

The site selected for life history studies was the Meramec River at the University of Missouri Hugo Wurdack Research Farm in

Crawford County, Missouri. At this locality, the Meramec River is a 5th order stream located in the Meramec River Basin, which is in the Ozark Plateau physiographic region. The study area is within a cleared/grazed land use area with a narrow, natural riparian zone. Riffle habitats were quantitatively sampled with a Surber sampler, which has a base area of 30.5 × 30.5 cm (1 ft^2) and mesh openings of 1 mm. The sediment within the frame was disturbed to a depth of approximately 6 cm with a hand rake for approximately 1 min. Benthic invertebrates collected into the net of the sampler were transferred into bottles containing 80% ethyl alcohol and transported to the laboratory. The sampling regime for 1992–93 consisted of weekly collections of 40 samples from mid-May through late September, and monthly collections for the remainder of the sampling year, for a total of 28 sampling dates.

Instar determination of *Stenonema mediopunctatum* (McDunnough).—Of the mayfly nymphs collected, *S. mediopunctatum* occurred consistently and in large numbers, making it a candidate taxon for analysis of body measurements and instar determination. Nymphs of *S. mediopunctatum* from representative subsamples of 13 of the 28 collecting dates, from May through November 1992, were measured (mm) using an ocular micrometer. Measurements consisted of head length and width, body width, and lengths of the profemur, protibia, mesofemur, mesotibia, metafemur, and metatibia. Head capsule was measured at the greatest width, which included the compound eyes. Body width also was measured at its greatest width, which was the pronotum. A total of 322 specimens was examined.

Cluster analyses were performed for head capsule width separately, and for the entire data set, which included all nine mensural characters. Hierarchical methods of classification, or cluster analyses, operate on a matrix of similarities among a set of units. This technique places variables that are highly correlated and similar to each other into groups and excludes from clusters those variables that are unlike. With the average linkage method (UPGMA), the distance between two clusters is the average distance between pairs of observations, one in each cluster. This analytical technique is a commonly used tool in morphometric research (e.g., Strauss 1992, Wool and Manheim 1992).

Because shape change accounts for significant variation among instars of insects undergoing allometric development, the data were ln-transformed and all body measurements again were clustered. Only a single dimension can be examined with linear measurements; therefore, the ln-transformed values were used to provide a mathematical approximation of shape by calculating two-dimensional variables [$\ln A + \ln B = \ln (A * B)$].

After cluster analysis had grouped the individuals, first-level (most similar) groupings were assumed to represent instars and were numbered. These instar numbers were then assigned to all individuals in the original database. Discriminant function analysis (DFA) simultaneously maximizes intergroup differences and minimizes intragroup variation among individuals by altering the linear combination of variables on each of a number of orthogonal axes. DFA then was used to determine the degree of overlap among the clustered groups (instars), giving an indication of the range of variation within and among instar groupings. The subsequent classification phase of DFA then assigns each specimen to an instar based on the linear combination of variables from each discriminant function axis. Percent of correct assignments was used as a separate measure of morphometric distinction among instars. More specifically, percent correct classification was used to determine the accuracy of instar assignments. All statistical analyses were conducted using SPSS, version 4.0 (SPSS Inc., Chicago, IL).

Voltinism determination of lotic mayflies.—The four species chosen for analysis of voltinism were those with nymphs that were collected throughout the year: *S. mediopunctatum* (Heptageniidae), *Tricorythodes curvatus* Allen (Leptohyphidae), *Caenis hilaris* (Say), and *C. latipennis* Banks (Caenidae). A black light was used to collect adult mayflies, and was positioned near the stream 12 h prior to each collection period. Using an ocular micrometer, nymphal head capsule measurements were tabulated for approximately 600–700 specimens of each of the four species from 17 of the 28 collecting dates (May 1992 through April 1993). The number of specimens collected and measured per month for *S. mediopunctatum* ranged from 15 to 54, whereas *T. curvatus* ranged from 4 to 50, *C. hilaris* from 3 to 50, and *C. latipennis* from 6 to 50. Size-frequency histograms then were constructed to determine voltinism.

Results

Instar determination of *Stenonema mediopunctatum*.—Using cluster analysis, raw data of head capsule width measurements clustered into five distinct groups at a small dissimilarity distance (Fig. 1A). The DFA classification rate was 89.6% using this approach. When raw data of all mensural characters were clustered, the data again exhibited five distinct groups (Fig. 1B), however the DFA classification rate improved to 95.6%. Finally, ln-transformed data for all mensural characters clustered into nine groups (Fig. 1C), and still maintained a high level of DFA classification (95.3%), indicating very distinct groupings.

Voltinism determination of lotic mayflies.—*Stenonema mediopunctatum* showed mainly incosistent size-frequency distributions throughout the year, although some patterns can be observed (Fig. 2A). The largest head capsule width measurements occurred in May and April for 1992 and 1993, respectively, although the frequencies of these measurements were low. Numbers remained high throughout the growing season for this species, and adults emerged during June, July, and August.

Tricorythodes curvatus exhibited very small changes in size-frequency distributions throughout the year (Fig. 2B). From May through September, head capsule widths were as high as 1 mm; however, from October through April, the population was represented primarily by higher frequencies of smaller individuals. Adults of this species emerged during June, July, August, and September, and a second cohort can be observed in September.

Caenis hilaris was the least abundant of the four species examined, and no specimens were collected during December, January, February, or March (Fig. 2C). Peak abundance and the largest specimens occurred during early summer months (i.e., late April, May, June). *Caenis latipennis* exhibited a pattern similar to that of *C. hilaris*, with the largest sized individuals occurring in April and May (Fig. 2D). Adult emergence of both species of *Caenis* occurred during June through September.

Discussion

Analysis of mensural characters of *S. mediopunctatum* nymphs revealed that at least nine instars were present in the samples from the Meramec River during the study period. Because a single outlier existed in each analysis, representing an instar markedly dissimilar from the others, it is likely that the morphometric distribution does not represent a contiguous succession of instars. Using a suite of ln-transformed body measurements provided increased resolution over using non-transformed data and increased resolution and precision over using head capsule widths alone. Cluster analysis for head capsule widths resulted in five instars and the lowest classification rate (89.6%) of the three methods evaluated. Thus, a relatively high degree of overlap (11.4%) existed among groups (instars) when based on only a single variable. When a suite of nine mensural variables was used, the number of instars remained unchanged, although classification accuracy increased dramatically to 95.6%. Thus, the number of

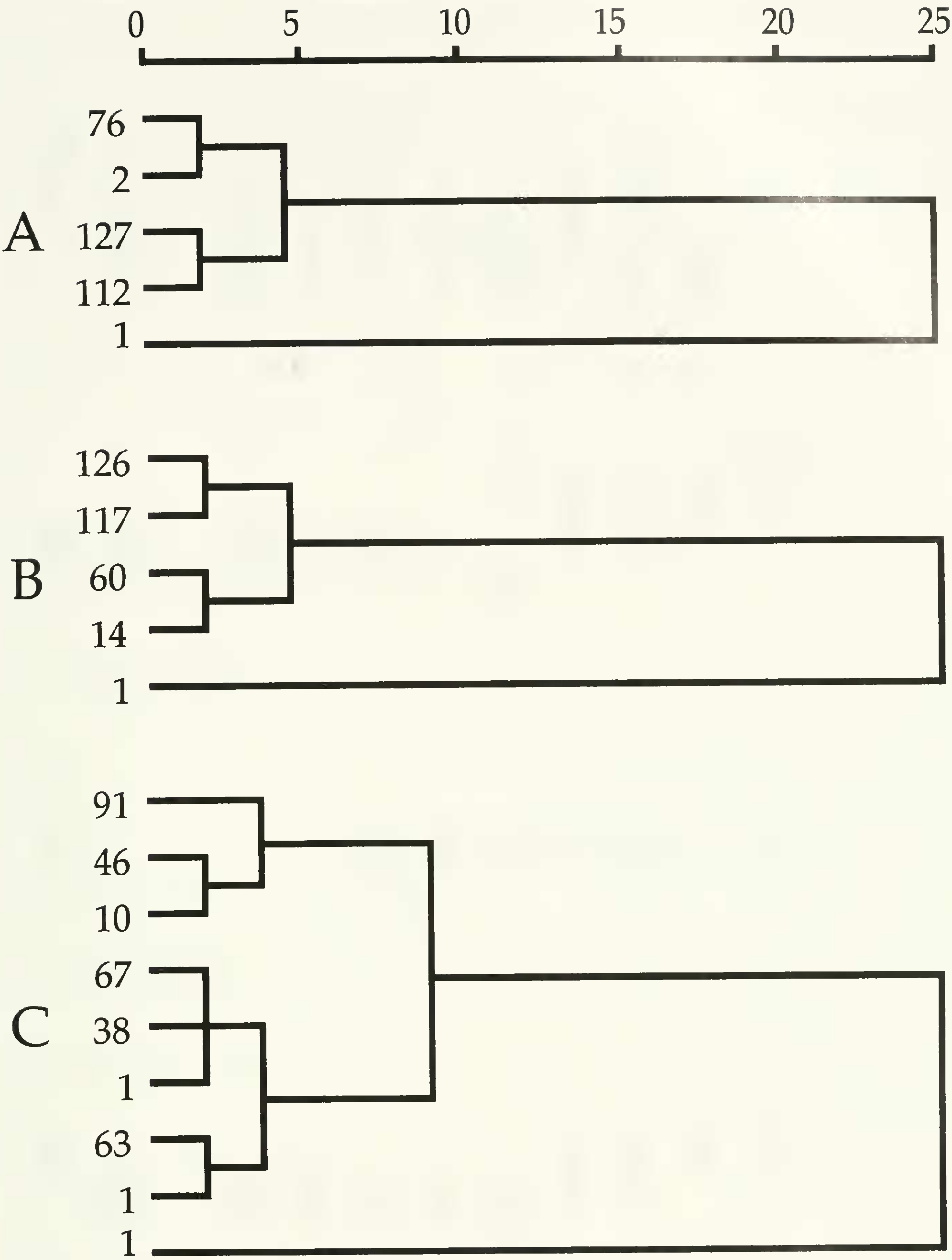

Fig. 1. Dendrogram of cluster analysis of *Stenonema mediopunctatum* nymphs based on measurements of (A) head capsule widths, (B) raw data of nine body characters, and (C) ln-transformed data of nine body characters. Numbers on the left of each dendrogram indicate the number of individuals within a given cluster.

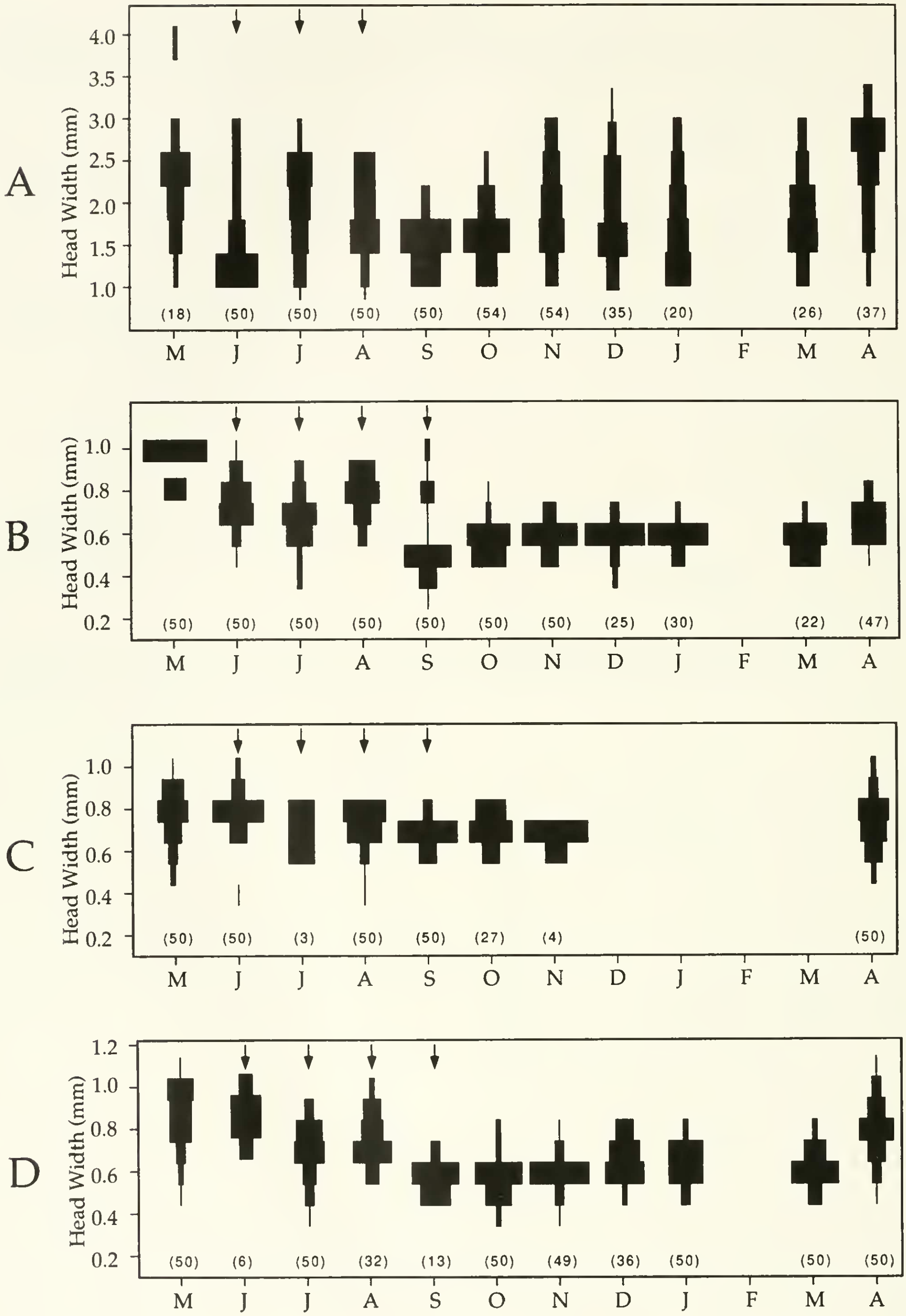

Fig. 2. Percent of individuals in each head capsule size class within each sample of four species of Ephemeroptera collected from the Meramec River from March 1992 through April 1993. A) *Stenonema mediopunctatum*, B) *Tricorythodes curvatus*, C) *Caenis hilaris*, D) *Caenis latipennis*. Arrows indicate adult presence in black light samples, numbers in parentheses indicate the number of immatures measured.

misclassifications was approximately halved while maintaning the same level of grouping resolution. The use of ln-transformed variables provided dramatically increased discrimination among groups, and cluster analysis identified nine groups. All characters were highly correlated with DFA axis 1 and accounted for >95% of the variation for both raw data and ln-transformed data. Thus, all characters measured were important in discriminating among instars. Although the resolving power of the ln-transformed data set nearly doubled the number of discernible groups, classification accuracy remained high at 95.3% (i.e., 4.7% of cases were assigned to the incorrect group). Clearly, the possibility exists that additional instars exist that were not discerned. Nonetheless, by using a suite of ln-transformed variables, this method of instar determination represents a significant improvement over the commonly used head capsule width analysis because both resolving power and precision are substantially increased.

Insects with a small number of distinct instars may have greater inter-instar variance in size than intra-instar variance, which allows for the number of instars to be effectively discerned using techniques such as the multivariate analysis of body measurement presented here. For example, Sites (1991), using such methods, confirmed that *Pelocoris poeyi* (Guerin Meneville) (Heteroptera: Naucoridae) has five nymphal instars. In mayflies, overall increase in size of the nymph occurs at each molt as well as a differential growth rate of body parts (allometric growth); therefore, one function of molting is to change morphological structure (Ide 1935). Although the method discussed here was shown to improve our ability to discriminate among instars from a field population, laboratory rearing of mayflies would provide the most accurate data, although environmental influences on body size and shape and instar number would not be realized.

Population studies of species of *Stenonema* have revealed mainly univoltine winter cycles, which are characterized by a single generation overwintering in the nymphal stage (Clifford 1982). The pattern observed during this research for *S. mediopunctatum* approximates this pattern, with overwintering nymphs, continued growth throughout spring, and emergence in early summer. The wide variation in frequency values and inconsistent patterns most likely indicate a large number of nymphal instars present in the stream at a given time.

Temperate populations of *Tricorythodes* generally have been characterized as multivoltine (Clifford 1982). Although some temporal size shifts were observed during this study, a pattern was not clear. Because multivoltine life history patterns typically would be evidenced by a wide range of sizes of individuals throughout the year, *T. curvatus* apparently also is multivoltine in this region.

Life histories of many *Caenis* populations have been reported to be quite flexible (Clifford 1982). Nearly half of the *Caenis* species for which life histories have been documented are univoltine winter and half are bivoltine winter-summer, with an overwintering generation in the nymphal stage and one summer generation (Clifford 1982). For both *Caenis* species examined here, the largest individuals occurred from late April through mid-June, with a summer emergence period over four months (June–September). Therefore, it appears that both *C. hilaris* and *C. latipennis* have a univoltine life history with an overwintering nymphal stage. Black light collections of adults of these species also support univoltinism.

Geographic variation in voltinism due to thermal or nutritional regimes commonly has been reported (Sweeney 1984); however, even within populations, variability in life cycle duration can exist (Butler 1984). Therefore, because of the potential for environmentally induced variation that exists for the majority of aquatic insects (Wallace and Merritt 1980), life history attributes, such as instar number and voltinism, should not necessarily be considered species specific traits, but should be examined on a regional basis for each population. Larger sample sizes may have improved resolution in the size-frequency graphs, although a high degree of variation is inherent, and should be expected, in mayfly populations.

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